INFECTION AND IMMUNITY, Mar. 2006, p. 1528–1536 Vol. 74, No. 3
0019-9567/06/$08.00+0 doi:10.1128/IAI.74.3.1528–1536.2006


# The Onset of CD8$^+$-T-Cell Contraction Is Influenced by the Peak of *Listeria monocytogenes* Infection and Antigen Display

Brandon B. Porter[1] and John T. Harty[2]*

*Interdisciplinary Program in Immunology[1] and Department of Microbiology,[2] University of Iowa, Iowa City, Iowa 52242*

Received 6 August 2005/Returned for modification 22 September 2005/Accepted 14 December 2005

**The CD8$^+$-T-cell response to infection with *Listeria monocytogenes* consists of expansion, contraction, and memory phases. The transition between expansion and contraction is reported to occur on different days postinfection with virulent (8 to 9 days) and attenuated (Δ*actA*) (7 days) *L. monocytogenes* strains. We hypothesized that differences in the infectious courses, and therefore antigen (Ag) display, determine the precise time of the expansion/contraction transition in response to these infections. To test this, we infected BALB/c mice with 0.1 50% lethal dose of Δ*actA* or virulent *L. monocytogenes* and measured bacterial numbers, Ag display, and Ag-specific CD8$^+$-T-cell responses on various days after infection. We found that bacterial numbers and Ag display peaked between 12 and 36 h and between 36 and 60 h after infection with Δ*actA* and virulent *L. monocytogenes* strains, respectively. Infection with Δ*actA L. monocytogenes* resulted in a sharp peak in the Ag-specific CD8$^+$-T-cell response on day 7, while infection with virulent *L. monocytogenes* yielded a prolonged peak with equivalent numbers of Ag-specific CD8$^+$ T cells on days 6, 7, and 8 after infection. Truncating virulent infection with antibiotics on day 1 or 2 after infection resulted in a shift in the expansion/contraction transition from day 8 to day 7 after infection. However, antibiotic treatment beginning on day 3, after the peak of virulent *L. monocytogenes* infection and Ag display, had no effect upon the magnitude or timing of the CD8$^+$-T-cell response. These results demonstrate a direct relationship between the course of infection and Ag display and that the timing of these events is important in shaping the T-cell response to infection.**

The CD8$^+$-T-cell response to bacterial and viral pathogens is important in clearing infections and establishing long-term immunity. In order to initiate a CD8$^+$-T-cell response after acute infection, dendritic cells (DC) must process and present pathogen-derived antigen (Ag) in the context of major histocompatibility complex class I and costimulatory molecules to naïve CD8$^+$ T cells (22, 26). The Ag-specific CD8$^+$ T cells are thereby activated to massively expand in number, differentiate into effector cells, and disseminate throughout the body (12, 33). Anywhere from 5 to 10 days after acute infection, the number of Ag-specific T cells reaches a peak and then begins to contract to stable memory levels (11, 12, 15, 19, 33, 37, 38). The pattern of the CD8$^+$-T-cell response to infection (expansion in numbers followed by contraction to stable memory levels) is very consistent within a number of acute infections of mice, including lymphocytic choriomeningitis virus (LCMV), *Listeria monocytogenes*, herpes simplex virus type 1, *Plasmodium yoelii*, and influenza virus infections (1, 11, 12, 15, 19, 33, 37, 38). After contraction, the resulting number of memory CD8$^+$ T cells generally represents 5 to 10% of the T-cell numbers at the peak of expansion (1). This numerical relationship between the magnitude of expansion and the number of memory cells is important because the number of memory cells determines the level of protection from subsequent infections (2, 40). It is not yet known what regulates the onset of this precisely controlled death of effector T cells.

More is known about the combination of factors that determine the magnitude of CD8$^+$-T-cell expansion. These factors are thought to include the number of available naïve Ag-specific precursors, the number of precursors that are recruited, the number of divisions, the amount of antigen produced by the organism, the level of Ag presentation by DC, the infectious dose, and the duration of infection (4, 8, 18, 21, 30, 39, 48, 49, 53). A multitude of recent studies highlight the notion that CD8$^+$-T-cell expansion is largely regulated at a very early stage of T-cell activation. For instance, only 2 to 2.5 h of antigenic stimulation in vitro was required to initiate a series of divisions in naïve CD8$^+$ T cells, and 24 h of stimulation could support differentiation of naïve Ag-specific T cells into effector and memory cells (23, 43, 51). In vivo, abbreviating the course of bacterial infections with antibiotics had little influence upon the pattern of a CD8$^+$-T-cell response or the generation of memory (4). For instance, when mice were infected with virulent or attenuated (Δ*actA*) *Listeria monocytogenes* and treated with antibiotics 24 h after infection, the magnitude of CD8$^+$-T-cell expansion was robust and decreased by only two- to fivefold compared to mice that did not receive antibiotics (4, 13, 32). A similarly modest reduction in the magnitude of the CD8$^+$-T-cell response was found in mice that received an abbreviated infection with herpes simplex virus type 1 (39). Thus, Ag-dependent programming of T-cell expansion and differentiation into effector and memory cells can occur in a short period of time; however, full T-cell expansion appears to require longer infection periods.

The data suggest that the programming model of CD8$^+$-T-cell expansion also applies to the contraction phase of the CD8$^+$-T-cell response. Antibiotic treatment of mice 24 h after infection with Δ*actA L. monocytogenes* resulted in timing and magnitude of CD8$^+$-T-cell contraction similar to those in mice that received Δ*actA L. monocytogenes* without antibiotic treat-

* Corresponding author. Mailing address: Department of Microbiology, University of Iowa, 3-512 Bowen Science Building, 51 Newton Road, Iowa City, IA 52242. Phone: (319) 335-9720. Fax: (319) 335-9006. E-mail: john-harty@uiowa.edu.

ment (4, 5). In addition, Ag-specific $CD8^+$ T cells in wild-type mice chronically infected with LCMV clone 13 and perforin knockout mice chronically infected with LCMV Armstrong underwent a normal initial contraction phase (4, 31, 42, 46). These experiments suggested that the timing of T-cell contraction could be separated from the timing of infection clearance and Ag withdrawal. However, while Δ*actA L. monocytogenes* yielded a $CD8^+$-T-cell response that reproducibly peaked 7 days postinfection regardless of the dose or infection duration (4, 5), multiple laboratories found that the $CD8^+$-T-cell response (to the same Ag) peaks between 8 and 9 days postinfection with 0.1 50% lethal dose ($LD_{50}$) of virulent *L. monocytogenes* (10, 13, 24, 32, 37). One difference between virulent and Δ*actA L. monocytogenes* infections is the time course of infection (14, 16, 29, 34, 52). Thus, we hypothesized that these differences in infection kinetics lead to differences in Ag presentation and the timing of $CD8^+$-T-cell contraction. Experimental support for this hypothesis could affect the T-cell programming model by demonstrating a relationship between the timing of Ag presentation and $CD8^+$-T-cell contraction.

In this report, we demonstrate that virulent *L. monocytogenes* infection results in delayed peaks in the bacterial load and Ag presentation compared to infection with Δ*actA L. monocytogenes*. In turn, these differences are associated with a prolonged transition between Ag-specific $CD8^+$-T-cell expansion and contraction after virulent *L. monocytogenes* infection compared to Δ*actA L. monocytogenes* infection. We also demonstrate that abbreviating virulent *L. monocytogenes* infection with antibiotics on day 1 or 2 after infection decreases the magnitude of the $CD8^+$-T-cell response and shifts the onset of T-cell contraction to an earlier time. However, antibiotic treatment on day 3 has no measurable influence upon the magnitude and timing of the $CD8^+$-T-cell response. Thus, on a population basis, the onset of the $CD8^+$-T-cell contraction program is influenced by the peak of infection and Ag presentation.

## MATERIALS AND METHODS

**Mice.** The BALB/c mice (Thy1.2$^+$ *H-2*$^d$) were from the National Cancer Institute (Frederick, MD). BALB/c Thy1.1$^+$ mice, provided by R. Dutton (Trudeau Institute, Saranac Lake, NY), were maintained by brother-sister mating under specific-pathogen-free conditions. Pathogen-infected mice were housed under the appropriate biosafety conditions. All mice were used at 6 to 16 weeks of age. L9.6 mice, provided by E. Pamer (Memorial Sloan Kettering, New York, NY), express a transgenic T-cell receptor (TCR) specific for the *H-2K*$^d$-restricted *L. monocytogenes* Ag $p60_{217-225}$ (27).

**Ab and peptides.** The following monoclonal antibodies (Ab) were used: phycoerythrin–anti-gamma interferon (IFN-γ) or allophycocyanin (APC)–anti-IFN-γ (clone XMG 1.2; eBioscience, San Diego, CA), fluorescein isothiocyanate–anti-CD8 or APC–anti-CD8 (clone 53-6.7; Pharmingen, San Jose, CA), fluorescein isothiocyanate–anti-Thy1.2 or APC–anti-Thy1.2 (clone 53-2.1; Pharmingen), phycoerythrin–anti-tumor necrosis factor alpha (TNF-α) (clone MP6-XT22; Pharmingen), and anti-FcRII (clone 2.4G2). Synthetic peptides, which represented the defined *L. monocytogenes* $LLO_{91-99}$ and $p60_{217-225}$ *H-2K*$^d$-restricted epitopes, were synthesized at Bio-Synthesis Inc., Lewisville, Texas.

**Bacterial infection of mice.** The virulent *L. monocytogenes* strain 10403s (6) ($LD_{50}$, $\sim 1 \times 10^4$ organisms) and the attenuated *L. monocytogenes* strain DP-L1942 (Δ*actA*; $LD_{50}$, $\sim 1 \times 10^7$ organisms) (9) were resistant to streptomycin and were grown, injected, and quantified as described previously (20, 41). The numbers of CFU per spleen were determined on various days after infection by harvesting the spleen in RPMI 1640 supplemented with 10% fetal calf serum and L-glutamine (RP10) without antibiotics. One milliliter of the dissociated spleen was centrifuged for 10 min at $16 \times 10^3 \times g$, the supernatant was discarded, the pellet was resuspended in 200 μl 0.2% IGEPAL CA-630 (Sigma) and $H_2O$, and serial dilutions were plated on streptomycin–3% trypticase soy broth plates. The limit-of-detection scores were as indicated in the figures. In the indicated experiments, 2 mg/ml ampicillin (Amp) (Na salt) was provided to the mice in their drinking water on different days after infection. The mice were treated with ampicillin for 2 to 3 days.

**Quantification of Ag-specific $CD8^+$ T cells.** The magnitude of the epitope-specific $CD8^+$-T-cell response was determined by peptide-stimulated intracellular-cytokine staining (ICS) for IFN-γ as described previously (5). Synthetic peptides were used at a final concentration of 200 nM. The percentages of IFN-$\gamma^+$ $CD8^+$ T cells in unstimulated samples from each mouse were subtracted from the peptide-stimulated value to determine the percentage of Ag-specific $CD8^+$ T cells. The total number of epitope-specific $CD8^+$ T cells per spleen was calculated from the percentage of IFN-$\gamma^+$ $CD8^+$ T cells, the percentage of $CD8^+$ T cells in each sample, and the total number of cells per spleen.

**CFSE labeling.** Splenocytes or cytotoxic-T-lymphocyte (CTL) lines were washed once in cold phosphate-buffered saline (PBS) and then resuspended at a concentration of $5 \times 10^6$ cells/ml in 1.5 μM carboxyfluorescein diacetate succinimidyl diester (CFSE) or 0.5 μM CFSE in PBS for splenocytes and CTL lines, respectively (45). The cells were incubated in a 37°C water bath for 10 min, and then fetal calf serum was added to a final concentration of 10% to inactivate the free CFSE. Splenocytes were washed three times with PBS and resuspended in isotonic saline at the desired concentration for injection into animals. After CFSE labeling, CTL lines were underlaid with 2 ml lympholyte M (Cedarlane Laboratories Ltd., Hornby, Ontario, Canada) and centrifuged at $1{,}600 \times g$ for 15 min, and then the cells at the interphase were harvested, washed three times in RP5, and resuspended in RP10. Ninety-five percent of the recovered $CSFE^+$ cells were $CD8^+$.

**DEAD assay.** The direct ex vivo antigen detection (DEAD) assay was performed with some modifications (4). The generation and maintenance of Ag-specific $CD8^+$-T-cell lines was as described previously (20). CFSE-labeled Ag-specific $CD8^+$ T cells ($3 \times 10^4$; day 5 or 6 after in vitro stimulation) were mixed with $3 \times 10^6$ splenocytes obtained at various times after infection from *L. monocytogenes*-infected mice. The cells were incubated for 6 h in the presence of 2 μl/ml GolgiPlug (Pharmingen) and 50 μg/ml α-interleukin 12 (IL-12) monoclonal antibody (C17.8). α-IL-12 monoclonal Ab was used to prevent cytokine-mediated IFN-γ production. CFSE-labeled cells were analyzed by intracellular cytokine staining for IFN-γ and TNF-α production. The results are reported as the percentage of $CFSE^+$ cells that produced cytokines. Cells from spleens were measured in triplicate and averaged; these averages were used to determine the group average and standard error of the mean. $CD8^+$ T cells incubated with naïve splenocytes in the presence or absence of various concentrations of synthetic peptides served as positive and negative controls in the assay.

**In vivo "functional" (DC) Ag presentation assay.** Before or on various days after infection, $1 \times 10^6$ CFSE (28)-labeled naïve L9.6 (Thy1.2) cells (determined by CD8α and Thy1.2 staining of whole L9.6 splenocytes) were intravenously transferred into naïve and infected BALB/c Thy1.1$^+$ mice. The BALB/c Thy1.1$^+$ mice were infected with 0.1 $LD_{50}$ of *L. monocytogenes* strain 10403s or DP-L1942. The spleens were harvested 2 days after transfer, and CFSE dilution of donor TCR transgenic T cells was determined by flow cytometry. The percentages of L9.6 cells that were CFSE low (daughter cells) are presented throughout the figures. This number increases with higher levels of "functional" Ag display.

## RESULTS

**Kinetics of *L. monocytogenes*-specific $CD8^+$-T-cell response after virulent and Δ*actA L. monocytogenes* infection.** In various reports, the peak of $CD8^+$-T-cell expansion in response to virulent *L. monocytogenes* varies from day 8 to day 9 postinfection (10, 13, 24, 32, 37). In contrast, we find that after infection with Δ*actA L. monocytogenes*, the $CD8^+$-T-cell response reproducibly peaks on day 7 (4). To determine if these differences were the result of *L. monocytogenes* infection kinetics or simply due to laboratory-to-laboratory variability, we first infected BALB/c mice, side by side, with ~0.1 to 0.2 $LD_{50}$ of virulent or Δ*actA L. monocytogenes* organisms and followed the $LLO_{91-99}$ and $p60_{217-225}$ Ag-specific $CD8^+$-T-cell responses. We found that infection with either strain of *L. monocytogenes* resulted in peak numbers of Ag-specific $CD8^+$ T cells on days 6 and 7 after infection (Fig. 1A). On day 7, virulent *L.*

*monocytogenes* infection resulted in $1.65 \times 10^6 \pm 9.62 \times 10^4$ Ag-specific cells, and Δ*actA L. monocytogenes* infection resulted in $1.41 \times 10^6 \pm 2.15 \times 10^5$ Ag-specific cells; representative fluorescence-activated cell sorter (FACS) analysis plots are displayed in Fig. 1B. In mice infected with Δ*actA L. monocytogenes*, there was a significant drop, 46.2% ($P = 0.0161$), in Ag-specific $CD8^+$-T-cell numbers on day 8 after infection (Fig. 1A). In contrast, after virulent *L. monocytogenes* infection, a significant drop, 36.6% ($P = 0.0336$), from the day 7 peak numbers did not occur until day 9 postinfection. Thus, virulent *L. monocytogenes* infection resulted in a prolonged transition between expansion and contraction in the Ag-specific $CD8^+$-T-cell response compared to Δ*actA L. monocytogenes* infection. This relationship is best demonstrated when the T-cell responses to each *L. monocytogenes* strain are normalized to day 7 levels of Ag-specific $CD8^+$ T cells (Fig. 1C). These results demonstrate that after infection of mice with similar relative $LD_{50}$s of Δ*actA* or virulent *L. monocytogenes*, the variations in the timing of $CD8^+$-T-cell contraction depend upon the *L. monocytogenes* strain and not laboratory-to-laboratory variability.

**Kinetics of virulent and Δ*actA L. monocytogenes* infection.** To ascertain how the kinetics of the $CD8^+$-T-cell response relate to the kinetics of infection, bacterial numbers (CFU) were determined in the spleens of mice infected with 0.1 to 0.2 $LD_{50}$ of virulent and Δ*actA L. monocytogenes* organisms on the indicated days after infection (Fig. 1D). At 12 hours postinfection, mice infected with $2.6 \times 10^6$ Δ*actA L. monocytogenes* organisms had 100 times more viable bacteria in their spleens than the mice infected with $1.2 \times 10^3$ virulent *L. monocytogenes* organisms. This early difference in infectious load was likely the result of a >1,000-fold difference in infecting doses. The timing of the infectious courses also differed. Splenic CFU reached a peak at 12 h after infection with Δ*actA L. monocytogenes*, while virulent *L. monocytogenes* infection peaked between days 1.5 and 2.5 after infection. The number of Δ*actA L. monocytogenes* organisms was at the limit of detection, 40 CFU, in the spleens of mice on day 3.5 after infection, whereas virulent *L. monocytogenes* organisms were measurable in the spleens of most mice until day 6.5 postinfection. These data are consistent with previous studies that utilized each *L. monocytogenes* strain individually (4, 52). The results demonstrate that infection with 0.1 to 0.2 $LD_{50}$ of Δ*actA L. monocytogenes* organisms yields a large bolus of bacteria during the first hours of infection with a relatively quick splenic clearance, while infection with 0.1 to 0.2 $LD_{50}$ of virulent *L. monocytogenes* organisms yields a delayed peak in bacterial load and delayed bacterial clearance.

**Kinetics of total Ag presentation after infection with virulent and Δ*actA L. monocytogenes* strains.** Our finding, described above, that the peaks of infection after Δ*actA* and virulent *L. monocytogenes* infection occur on different days suggests that there may be differences in the timing of Ag presentation. Considering the notion that early events dictate the magnitude and timing of the T-cell response, differences in Ag presentation kinetics might explain the prolonged transition between Ag-specific $CD8^+$-T-cell expansion and contraction found in mice infected with virulent *L. monocytogenes*.

We employed a DEAD assay as our first method of measuring Ag presentation. In this assay, we utilized $CD8^+$-T-cell lines specific for *L. monocytogenes* antigens as indicator cells and an *Escherichia coli* β-galactosidase (β-Gal)-specific $CD8^+$-T-cell line as a specificity control (4). In the presence of brefeldin A, CFSE-labeled $CD8^+$-T-cell lines are incubated with splenocytes from infected mice or naïve mice as a negative control. The frequency of CFSE-labeled $CD8^+$ T cells that are stimulated to produce IFN-γ and TNF-α, as detected by intracellular cytokine staining, represent a direct ex vivo measure of Ag display. The T-cell lines we used were previously activated and did not require costimulation for activation; as a result, the DEAD assay measures Ag presentation by any cell type that expresses the cognate peptide major histocompatibility complex (total Ag display). Subsequent to our first description of this assay (4), under some conditions we found nonspecific activation of $CD8^+$-T-cell lines. Doubling the concentration of brefeldin A, adding 50 μg/ml anti-IL-12 Ab, and abbreviating the incubation period from 12 h to 6 h eliminated this background. We used these conditions in the current studies. Importantly, the percentage of the indicated T cells that make IFN-γ and TNF-α increases with the concentration of cognate Ag (Fig. 2A) and also increases with the number of peptide-loaded splenocytes within the total splenocyte population (data not shown), thus demonstrating that this assay is dose responsive.

Mice were infected with ~0.5 $LD_{50}$ of Δ*actA* or virulent *L. monocytogenes*, and then Ag levels for two well-characterized *L. monocytogenes* Ags, $LLO_{91\text{-}99}$ (36) and $p60_{217\text{-}225}$ (35), were measured in the spleens at various times after infection. Naïve splenocytes were analyzed as a negative control. Spleens from three mice per condition were each measured for Ag in triplicate. Representative assays are displayed in Fig. 2B. Total $LLO_{91\text{-}99}$ and $p60_{217\text{-}225}$ Ag levels reached a peak between days 0.5 and 1.5 after Δ*actA L. monocytogenes* infection, while Ag levels peaked between days 1.5 and 2.5 after virulent *L. monocytogenes* infection. The combined results of two experiments are displayed in Fig. 2C. Infected splenocytes from virulent *L. monocytogenes*- and Δ*actA L. monocytogenes*-infected mice never activated the β-Gal-specific T-cell line above the response to naïve splenocytes, thus demonstrating that the $LLO_{91\text{-}99}$ and $p60_{217\text{-}225}$ results are Ag specific (Fig. 2C). Also, the T-cell lines generated similar peptide dose responses on each day, demonstrating that changes in sensitivity of the indicator cells did not account for the observed changes in IFN-γ production (data not shown). These results demonstrate that, similar to the infectious courses, Δ*actA L. monocytogenes* infection yields a peak in total Ag presentation earlier than virulent *L. monocytogenes* infection.

**Kinetics of "functional" Ag presentation after virulent and Δ*actA L. monocytogenes* infection.** The DEAD assay utilizes a T-cell population that can respond to cognate Ag without costimulation. The major benefit of this approach is that it provides a snapshot of total Ag levels as a result of the short incubation period. However, while depletion of $CD11c^+$ cells from the splenocyte population with magnetic beads significantly decreased Ag levels (reference 13 and data not shown), the DEAD assay is not a specific measure for Ag display on activated dendritic cells; the most relevant antigen-presenting cell for naïve-T-cell activation (22). We therefore decided to use a complementary in vivo method of measuring Ag display

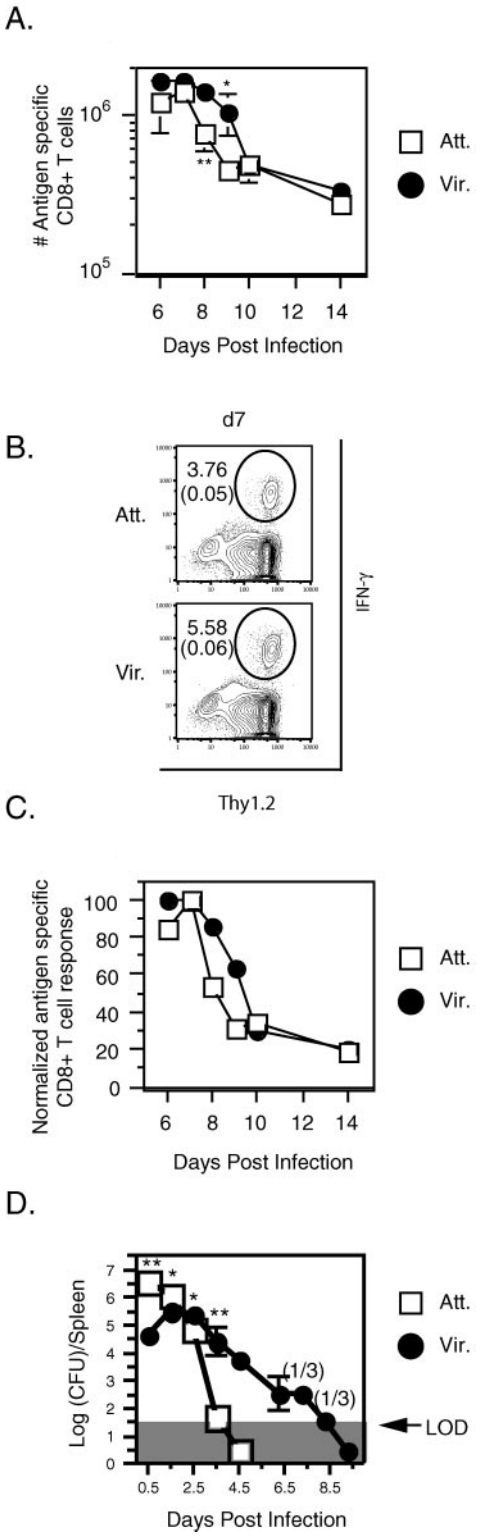


FIG. 1. The CD8+-T-cell response expansion/contraction transition is prolonged in virulent *L. monocytogenes* infection. BALB/c mice were infected with $2.6 \times 10^6$ Δ*actA L. monocytogenes* organisms (Att.) or $1.2 \times 10^3$ 10403s *L. monocytogenes* organisms (Vir.). (A) The total numbers of $LLO_{91-99}$- plus $p60_{217-225}$-specific CD8+ splenocytes were measured on the indicated days (d) after infection using ICS for IFN-γ. The first days of significant Ag-specific CD8+-T-cell decrease compared to day 7 postinfection are marked with asterisks. *, $P \leq 0.04$; **, $P \leq 0.02$. (B) Representative CD8+ gated FACS plots from representative mice 7 days after infection. The upper and lower numbers are the percentages of CD8+ cells that were IFN-γ positive in peptide-incubated or non-peptide-incubated splenocytes, respectively. (C) The total number of Ag-specific CD8+ T cells per spleen was normalized to the maximum response to each *L. monocytogenes* strain. (D) Portions of spleens from infected mice were plated on the indicated days after infection. LOD, limit of detection; the numbers in parentheses are the fractions of mice with measurable infection. Days with significant difference between Att. and Vir. infections: *, $P \leq 0.01$; **, $P \leq 0.001$. The data shown are means ± standard deviations of three mice per group and are representative of four or more experiments.

that utilized naïve TCR transgenic CD8+ T cells as the responder population (32).

To assess "functional" Ag display, we infected BALB/c Thy1.1 congenic mice with ~0.1 $LD_{50}$ of Δ*actA* or virulent *L. monocytogenes*. We then transferred $1 \times 10^6$ naïve CFSE-labeled Thy1.2-positive $p60_{217-225}$-specfic L9.6 TCR transgenic CD8+ T cells into infected and uninfected mice at different times before and during infection (52). Jung et al. demonstrated that CD8+ TCR transgenic T cells absolutely require CD11c+ cells (primarily DC) in order to divide in response to *L. monocytogenes* infection (22). To increase the resolution of "functional" Ag display after infection, CFSE dilution, as a measure of cell division, was quantified in these cells 48 h after transfer, as opposed to 4 days after transfer as used in the initial description of this assay (52). The results are presented as the percentage of transferred L9.6 cells that divided; therefore, high numbers represent high levels of "functional" $p60_{217-225}$ Ag presentation. Representative data are displayed in Fig. 3A. We found maximal "functional" Ag display between days 0.5 and 1.5 of transfer in Δ*actA L. monocytogenes* infection and between days 1.5 and 2.5 of transfer after virulent *L. monocytogenes* infection (Fig. 3B). In virulent *L. monocytogenes* infection, Wong and Pamer found the highest stimulation of L9.6 cells when transferred 1 day postinfection (52). The simplest explanation for this discrepancy is that their assay for "functional" Ag display was 4 days, while ours was a 2-day assay. This meant that, in their assay, transgenic T cells transferred on day 1 experienced the complete peak of "functional" Ag presentation, whereas transgenic T cells transferred on day 2 missed a period of peak "functional" Ag presentation and therefore produced fewer transgenic T cells that had divided. The shorter transfer period in our assay allowed for a more detailed analysis of the timing of "functional" Ag display. Our finding of a delayed peak in "functional" Ag display after virulent *L. monocytogenes* infection, compared to the peak in Δ*actA L. monocytogenes*-infected mice, was similar to the DEAD assay results and directly correlated with the infectious course of each *L. monocytogenes* strain. These data, combined with the notion that early events dictate the T-cell response, suggested that the comparatively delayed peak in Ag presentation in virulent-*L. monocytogenes*-infected mice induces the prolonged transition between expansion and contraction in the Ag-specific CD8+-T-cell response to virulent *L. monocytogenes*.

**Antibiotic treatment on the third day of infection has no measurable influence upon the timing of the Ag-specific CD8+-T-cell expansion/contraction transition.** As noted

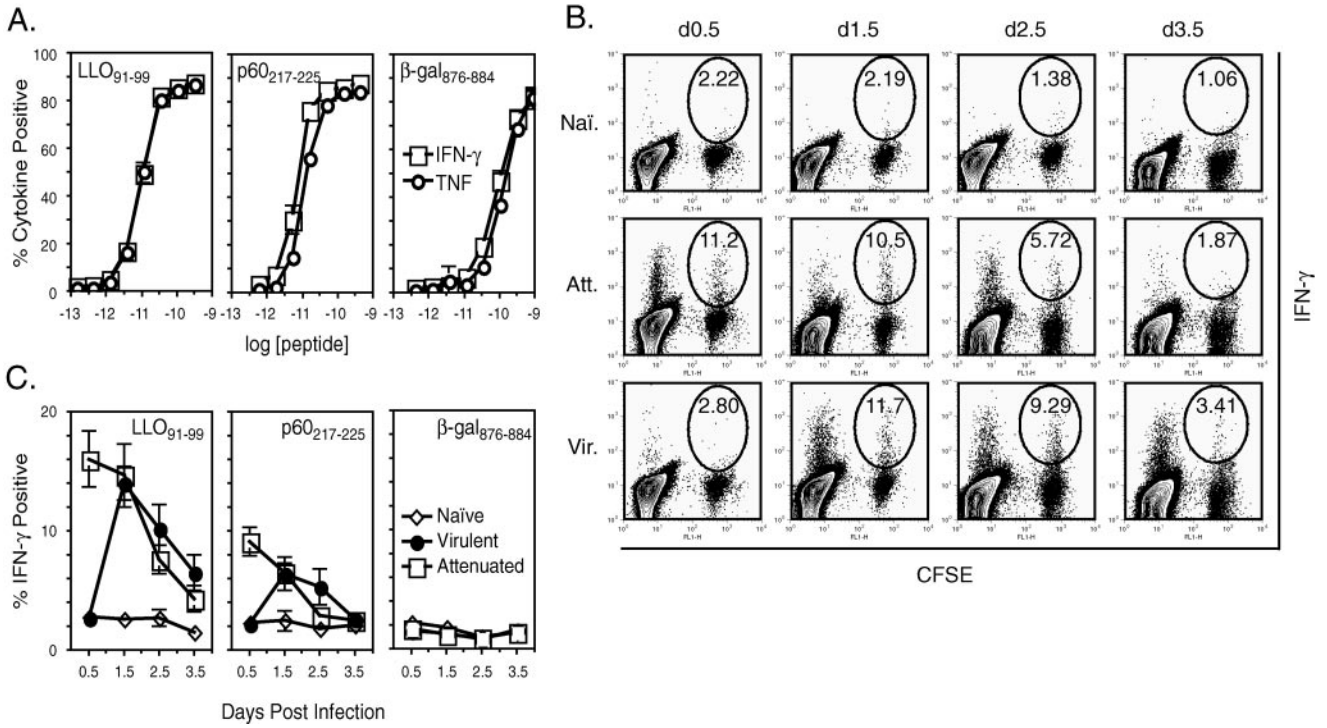


FIG. 2. Direct ex vivo Ag presentation after Δ*actA* and virulent *L. monocytogenes* infection. (A) The DEAD assay was performed with responder Ag-specific $CD8^+$-T-cell lines incubated with naïve splenocytes and increasing concentrations of cognate peptide. BALB/c mice were infected with $9.2 \times 10^6$ Δ*actA L. monocytogenes* organisms (Att.) or $8.8 \times 10^3$ 10403s *L. monocytogenes* organisms (Vir.). (B) Representative FACS plots from DEAD assays performed on infected or naïve splenocytes (Naï.) with a LLO91-99-specific T-cell line on the indicated days (d) after infection. The T-cell line was CFSE positive, and the numbers represent the percentages of CFSE-positive cells that were IFN-γ positive. (C) Composite DEAD assay results of two experiments using *L. monocytogenes*-specific T-cell lines and a negative-control, β-Gal-specific $CD8^+$-T-cell line. The data shown are means ± standard errors of the means for six mice per group.

above, there are significant differences in the courses of infection with either Δ*actA* or virulent *L. monocytogenes*. The peak of infection with Δ*actA L. monocytogenes* is between 0.5 and 1.5 days after infection, while the peak of infection with virulent *L. monocytogenes* is between 1.5 and 2.5 days after infection (Fig. 1D). To determine if the prolonged transition between expansion and contraction after virulent *L. monocytogenes* infection was the result of the delayed peak in infection, we measured

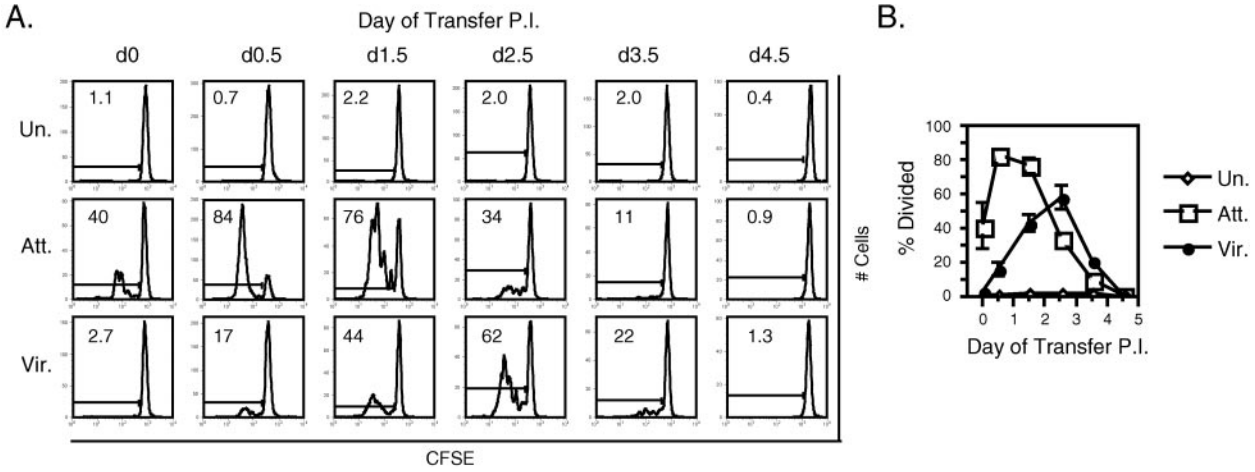


FIG. 3. In vivo "functional" Ag presentation after Δ*actA* and virulent *L. monocytogenes* infection. L9.6 cells ($1 \times 10^6$) were transferred into BALB/c Thy1.1 mice on the indicated days (d) after infection or no infection with $1.1 \times 10^6$ Δ*actA* (Att.) or $1.4 \times 10^3$ 10403s (Vir.) organisms. Spleens were harvested 48 h after transfer, and the $Thy1.2^+$ L9.6 cells were analyzed for CFSE dilution. (A) Representative histograms of L9.6 cells harvested from individual mice from uninfected (Un.) or infected mice. The numbers are the percentages of L9.6 cells that divided. (B) Average percentages of L9.6 cells that divided. The data shown are means ± standard deviations for three mice per group and are representative of two experiments.

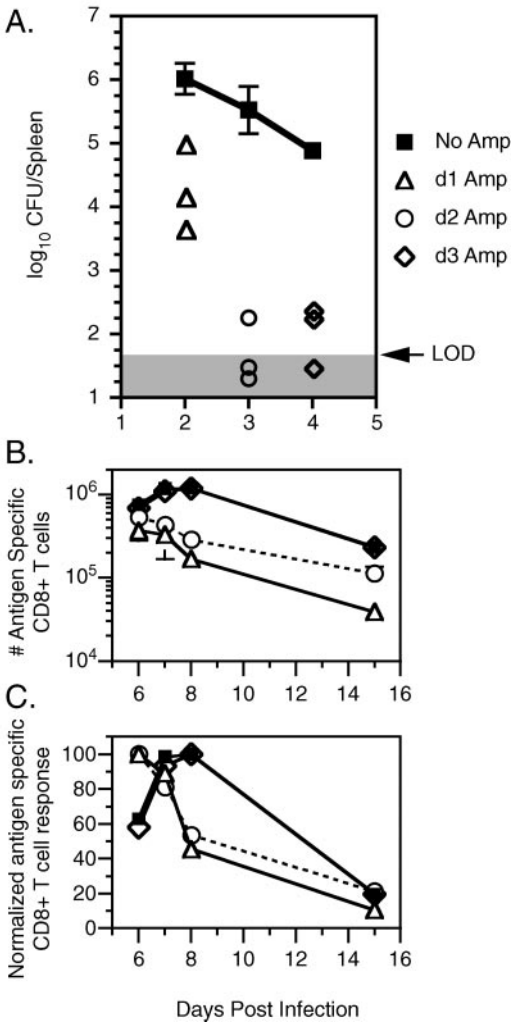


FIG. 4. Three days of infection are sufficient to shape the Ag-specific $CD8^+$-T-cell response. BALB/c mice were infected with $1.6 \times 10^3$ 10403s organisms (Vir.), and groups of mice were given ampicillin on different days (d) after infection. (A) Spleens from infected mice were plated 1 day after the initiation of Amp treatment, and CFU/spleen were determined. (B) Total numbers of $LLO_{91-99}$- and $p60_{217-225}$-specific $CD8^+$ T cells per spleen on the indicated days after infection, determined by ICS for IFN-γ. The data shown are means ± standard deviations for three mice per group and are representative of two experiments.

the Ag-specific $CD8^+$-T-cell response to virulent *L. monocytogenes* infection with or without antibiotic treatment on different days after infection. Wong and Pamer demonstrated that 24 h of antibiotic treatment substantially decreased "functional" Ag display in virulent-*L. monocytogenes*-infected mice (52).

We infected BALB/c mice with 0.1 $LD_{50}$ of virulent *L. monocytogenes* organisms. These mice were then split into four groups: one group did not receive antibiotic treatment; the others received an initial dose of 2 mg/ml Amp in their drinking water on day 1, 2, or 3 after infection. Analysis of CFU in the spleens 1 day after treatment revealed that Amp treatment was effective at limiting the infection on each of the days of treatment (Fig. 4A). Measurement of the Ag-specific $CD8^+$-T-cell response demonstrated that Amp treatment on days 1 and 2 postinfection shifted the onset of contraction to days 7 and 8 from day 9 (Fig. 4B and C). Also, the peak magnitudes of Ag-specific $CD8^+$-T-cell numbers were diminished from $1.20 \times 10^6 \pm 2.33 \times 10^5$ to $3.71 \times 10^5 \pm 8.63 \times 10^4$ and $5.33 \times 10^5 \pm 2.53 \times 10^5$ after Amp treatments on days 1 and 2 after infection, respectively (Fig. 4B). In contrast, Amp treatment on day 3 after infection had no discernible effect upon the magnitude of the response or the timing of the transition between expansion and contraction. These results demonstrate that at least 3 days of unmanipulated infection in BALB/c mice are required to induce a complete $CD8^+$-T-cell response to 0.1 $LD_{50}$ of virulent *L. monocytogenes* organisms. Also, these results reveal that earlier peaks in bacterial numbers result in Ag-specific $CD8^+$-T-cell contraction at an earlier time.

## DISCUSSION

In the current study, we utilized a comparison between Δ*actA* and virulent *L. monocytogenes* infections of BALB/c mice to investigate the relationships between the course of infection, Ag presentation, and the timing of Ag-specific $CD8^+$-T-cell contraction. Prior to this study, there were seemingly conflicting data concerning the relationships between the timing of infection and the timing of the $CD8^+$-T-cell response to *L. monocytogenes*. In the context of Δ*actA L. monocytogenes* infection, the magnitude of infection and the length of Ag display had little effect upon the timing of $CD8^+$-T-cell contraction (4). However, in virulent *L. monocytogenes* infection, the infection was prolonged and the peak of the $CD8^+$-T-cell response was delayed compared to reports of attenuated *L. monocytogenes* infection (4, 10, 14, 16, 29, 34, 52). One potential explanation for this discrepancy was that the timing of the peak of infection had more influence upon the onset of T-cell contraction than the total length of Ag display. Indeed, changing the dose of Δ*actA L. monocytogenes* or giving mice antibiotics 1 day after Δ*actA L. monocytogenes* infection had no influence upon the timing of the peak of infection (4). In contrast, we found that altering the peak of virulent *L. monocytogenes* infection with antibiotic treatment on day 1 or 2 after infection resulted in an earlier onset of $CD8^+$-T-cell contraction. In addition, antibiotic treatment after the peak of infection, day 3, had no discernible effect upon the magnitude or timing of the $CD8^+$-T-cell response. These results demonstrate that it is not the length of initial infection but the timing of the peak of infection and Ag display that determines the onset of $CD8^+$-T-cell contraction.

Similar results were found after *L. monocytogenes* infection of C57BL/6 mice. Antibiotic treatment before the peak of infection with a virulent recombinant *L. monocytogenes* strain that expressed ovalbumin (OVA), recombinant *L. monocytogenes*-OVA, resulted in an earlier OVA-specific $CD8^+$-T-cell peak (13). These results are in apparent conflict with a recent report that antibiotic treatment 24 h after virulent recombinant *L. monocytogenes*-OVA infection of C57BL/6 mice had no influence on $CD8^+$-T-cell expansion but decreased the generation of memory cells, particularly in tissues (50). However, these conclusions were based upon analysis of $CD8^+$-T-cell numbers on days 7 and 9 after infections, whereas Corbin and Harty demonstrated that the peak of the T-cell response to

recombinant *L. monocytogenes*-OVA in C57BL/6 mice is on day 8 after infection (13). In the present study, we would have concluded that antibiotic treatment decreases the capacity to create memory cells in response to virulent *L. monocytogenes* if we had confined our analyses of T-cell expansion to day 6, a period of similar expansion levels, and a later date when Ag-specific $CD8^+$-T-cell numbers contracted in all groups. While laboratory-to-laboratory variability could also account for the differences, the data underscore how daily analysis may be required to fully appreciate the transition between expansion and contraction in the T-cell response.

These experiments are not able to directly determine if the timing of the T-cell response is related to the timing of the peak in Ag presentation or the peak of inflammation, because antibiotic treatment likely decreases both. However, several laboratories have established that inflammation alone has little effect upon the timing of Ag-specfic $CD8^+$-T-cell contraction (10, 18). Badovinac et al. demonstrated that inflammation was necessary during $CD8^+$-T-cell activation for T-cell contraction to occur, but these studies provided no evidence that inflammation affected the timing of $CD8^+$-T-cell contraction (3). Hamilton and Harty immunized mice with peptide-coated DC, with or without infection with virulent *L. monocytogenes* that expressed or did not express the tested Ag (18). These experiments demonstrated that prolonging the inflammation in the absence of the test antigen had no influence upon the onset of $CD8^+$-T-cell contraction after DC-peptide immunization, whereas infection of DC-peptide-immunized mice with virulent *L. monocytogenes* that expressed the peptide resulted in a prolonged peak in test peptide-specific $CD8^+$ T cells. These experiments demonstrated that prolonging inflammation during the first 3 to 4 days of immunization had little influence upon the timing of the $CD8^+$-T-cell response. Similarly, Busch et al. found that superinfection of *L. monocytogenes*-infected mice with a high-dose Ag-expressing *L. monocytogenes* prolonged the expansion phase of Ag-specific $CD8^+$ T cells compared to superinfection with *L. monocytogenes* that lacked the Ag (10). Therefore, while inflammation is required for $CD8^+$-T-cell contraction, inflammation alone, early and late in the $CD8^+$-T-cell response, does not appear to influence the timing of $CD8^+$-T-cell contraction. In contrast, the timing of $CD8^+$-T-cell contraction can be affected by the timing of Ag display.

Perhaps the most interesting finding in the present study is that a drop in total and "functional" Ag presentation is reproducibly followed ~5 days later with an ~40 to 60% drop in the number of Ag-specific $CD8^+$ T cells. For example, after Δ*actA L. monocytogenes* infection, "functional" and total Ag displays decrease significantly between days 1.5 and 2.5 after infection. This is followed ~5 days later by an ~50% drop in the number of Ag-specific $CD8^+$ T cells, between days 7 and 8 after infection. After infection with virulent *L. monocytogenes*, total and "functional" Ag displays decrease between days 2.5 and 3.5 after infection, again followed by an ~40% decrease in numbers of Ag-specific $CD8^+$ T cells between days 8 and 9 after infection. Also, when mice are given antibiotics on day 2 after virulent *L. monocytogenes* infection, there is a significant drop in infection by day 3, and this is followed by an ~50% decrease in numbers of Ag-specific $CD8^+$ T cells between day 7 and day 8, again ~5 days after the decrease in infection.

This 5-day relationship between the drop in "functional" Ag display and the initiation of the contraction phase was also found in malaria infection of mice. Hafalla et al. demonstrated that the peak of "functional" Ag presentation after attenuated *P. yoelii* infection is between 0 and 8 h after infection, followed by a drop in "functional" Ag presentation by 24 h after infection (17). Sano et al. showed that, under similar conditions, the peak of the TCR transgenic $CD8^+$-T-cell response to attenuated *P. yoelii* infection, on day 5, is followed by an ~50% decrease in numbers of TCR transgenic $CD8^+$ T cells on day 6, ~5 days after the drop in "functional" Ag presentation (38). Together, these data suggest that the timing of Ag presentation by DC is a major determinant of the shape of the $CD8^+$-T-cell response curve and that $CD8^+$ T cells likely contain an internal timing mechanism that initiates the contraction program ~5 days after their last interaction with cognate Ag-laden DC.

Virulent and Δ*actA L. monocytogenes* infections progress with different kinetics and different mechanisms. Virulent *L. monocytogenes* organisms spread from cell to cell without access to the extracellular space, while Δ*actA L. monocytogenes* infection extends by growing within cells until the host cell bursts, exposing the now extracellular *L. monocytogenes* to neighboring cells (9, 14, 16, 25, 44). It is possible that these different mechanisms of bacterial spread lead to Ag presentation on different DC subsets or differential activation of DC, leading to the divergent timing of $CD8^+$-T-cell contraction in these infections. However, we do not favor this hypothesis because differences in the timing of virulent *L. monocytogenes* infection (antibiotic treatment) changed both the timing and magnitude of $CD8^+$-T-cell contraction.

A number of possible mechanisms exist for how the peak of acute infection might influence the timing of the peak in the $CD8^+$-T-cell response. One possibility is that the peak of infection, which is coordinate with the peak of "functional" Ag display, is the period when the most naïve Ag-specific $CD8^+$ T cells are recruited to respond to the infection. In this scenario, T cells are programmed to undergo a fixed number of divisions after stimulation, and the peak of the T-cell response is the result of those T cells that are activated at the peak of "functional" Ag display reaching their final division. Another possibility is that Ag presentation on DC continues to influence the division "program" of activated T cells, perhaps through upregulation of antiapopotic molecules through interactions with CD28, OX40L, 4-1BBL, and/or CD70 (30). Here, the peak of "functional" Ag display is the period when the highest percentage of activated T cells can interact one or more times with Ag in the context of DC. After the peak of "functional" Ag display, there is limited influence upon $CD8^+$-T-cell division and the population of Ag-specific $CD8^+$ T cells peaks ~5 days after their last interaction with Ag in the context of DC. Of course, it is possible for some combination of these mechanisms to contribute to the T-cell response.

It has not escaped us that $CD8^+$-T-cell contraction was observed in the presence of high titers of virus after overwhelming infections with chronic viruses (e.g., LCMV clone 13) (47). At first, this observation suggests that the timing of Ag withdrawal might not be related to the timing of $CD8^+$-T-cell contraction. However, LCMV clone 13 infects and kills DC within 7 days of infection (7). Therefore, it is possible that the peak of "functional" Ag display determines the timing of $CD8^+$-T-cell contraction, even under these conditions of

chronic Ag display on non-dendritic cells. Another possible explanation for CD8+-T-cell contraction in the presence of chronic Ag display is that all of the Ag-specific CD8+-T-cell precursors are recruited into the response within the first 3 to 4 days of infection and the recruited T cells undergo a fixed number of divisions until their contraction in numbers. We are investigating these possibilities.

In summary, our results demonstrate that the peak of *L. monocytogenes* infection and "functional" Ag display determines the onset of CD8+-T-cell contraction. These results provide additional support for the notion that there is a period of Ag-dependent programming of the CD8+-T-cell response, followed by antigen-independent expansion and contraction of Ag-specific CD8+-T-cell numbers. We also found that a drop in "functional" Ag display is reproducibly followed, ~5 days later, with contraction in CD8+-T-cell numbers. This relationship suggests that, while the length of Ag display is not immediately related to the timing of CD8+-T-cell contraction, a drop in "functional" Ag display initiates an internal 5-day clock for contraction of the Ag-specific CD8+-T-cell response.

## ACKNOWLEDGMENTS

We thank S. Perlman for critical review of the manuscript and for technical assistance. B. B. Porter also thanks his previous mentors, R. Bertrand, W. Arend, and C. Gabay.

This work was supported by NIH grants AI42767, AI46653, AI50073, and AI059752 to J.T.H. and T32AI07485 to B.B.P.

## REFERENCES

1. **Ahmed, R., and D. Gray.** 1996. Immunological memory and protective immunity: understanding their relation. Science **272:**54–60.
2. **Badovinac, V. P., K. A. Messingham, S. E. Hamilton, and J. T. Harty.** 2003. Regulation of CD8+ T cells undergoing primary and secondary responses to infection in the same host. J. Immunol. **170:**4933–4942.
3. **Badovinac, V. P., B. B. Porter, and J. T. Harty.** 2004. CD8+ T cell contraction is controlled by early inflammation. Nat. Immunol. **5:**809–817.
4. **Badovinac, V. P., B. B. Porter, and J. T. Harty.** 2002. Programmed contraction of CD8+ T cells after infection. Nat. Immunol. **3:**619–626.
5. **Badovinac, V. P., A. R. Tvinnereim, and J. T. Harty.** 2000. Regulation of antigen-specific CD8+ T cell homeostasis by perforin and interferon-gamma. Science **290:**1354–1358.
6. **Bishop, D. K., and D. J. Hinrichs.** 1987. Adoptive transfer of immunity to *Listeria monocytogenes*. The influence of in vitro stimulation on lymphocyte subset requirements. J. Immunol. **139:**2005–2009.
7. **Borrow, P. E., F. Claire, and M. B. Oldstone.** 1995. Virus-induced immunosuppression: immune system-mediated destruction of virus-infected dendritic cells results in generalized immune suppression. J. Virol. **69:**1059–1070.
8. **Bousso, P., J. P. Levraud, P. Kourilsky, and J. P. Abastado.** 1999. The composition of a primary T cell response is largely determined by the timing of recruitment of individual T cell clones. J. Exp. Med. **189:**1591–1600.
9. **Brundage, R. A., G. A. Smith, A. Camilli, J. A. Theriot, and D. A. Portnoy.** 1993. Expression and phosphorylation of the *Listeria monocytogenes* ActA protein in mammalian cells. Proc. Natl. Acad. Sci. USA **90:**11890–11894.
10. **Busch, D. H., K. M. Kerksiek, and E. G. Pamer.** 2000. Differing roles of inflammation and antigen in T cell proliferation and memory generation. J. Immunol. **164:**4063–4070.
11. **Busch, D. H., I. Pilip, and E. G. Pamer.** 1998. Evolution of a complex T cell receptor repertoire during primary and recall bacterial infection. J. Exp. Med. **188:**61–70.
12. **Butz, E. A., and M. J. Bevan.** 1998. Massive expansion of antigen-specific CD8+ T cells during an acute virus infection. Immunity **8:**167–175.
13. **Corbin, G. A., and J. T. Harty.** 2004. Duration of infection and antigen display have minimal influence on the kinetics of the CD4+ T cell response to *Listeria monocytogenes* infection. J. Immunol. **173:**5679–5687.
14. **Domann, E., J. Wehland, M. Rohde, S. Pistor, M. Hartl, W. Goebel, M. Leimeister-Wachter, M. Wuenscher, and T. Chakraborty.** 1992. A novel bacterial virulence gene in *Listeria monocytogenes* required for host cell microfilament interaction with homology to the proline-rich region of vinculin. EMBO J. **11:**1981–1990.
15. **Flynn, K. J., G. T. Belz, J. D. Altman, R. Ahmed, D. L. Woodland, and P. C. Doherty.** 1998. Virus-specific CD8+ T cells in primary and secondary influenza pneumonia. Immunity **8:**683–691.
16. **Goossens, P. L., and G. Milon.** 1992. Induction of protective CD8+ T lymphocytes by an attenuated *Listeria monocytogenes actA* mutant. Int. Immunol. **4:**1413–1418.
17. **Hafalla, J. C., G. Sano, L. H. Carvalho, A. Morrot, and F. Zavala.** 2002. Short-term antigen presentation and single clonal burst limit the magnitude of the CD8+ T cell responses to malaria liver stages. Proc. Natl. Acad. Sci. USA **99:**11819–11824.
18. **Hamilton, S. E., and J. T. Harty.** 2002. Quantitation of CD8+ T cell expansion, memory, and protective immunity after immunization with peptide-coated dendritic cells. J. Immunol. **169:**4936–4944.
19. **Harrington, L. E., R. Most, J. L. Whitton, and R. Ahmed.** 2002. Recombinant vaccinia virus-induced T-cell immunity: quantitation of the response to the virus vector and the foreign epitope. J. Virol. **76:**3329–3337.
20. **Harty, J. T., and M. J. Bevan.** 1995. Specific immunity to *Listeria monocytogenes* in the absence of IFN gamma. Immunity **3:**109–117.
21. **Hou, S., L. Hyland, K. W. Ryan, A. Portner, and P. C. Doherty.** 1994. Virus-specific CD8+ T-cell memory determined by clonal burst size. Nature **369:**652–654.
22. **Jung, S., D. Unutmaz, P. Wong, G. Sano, K. De los Santos, T. Sparwasser, S. Wu, S. Vuthoori, K. Ko, F. Zavala, E. G. Pamer, D. R. Littman, and R. A. Lang.** 2002. In vivo depletion of CD11c+ dendritic cells abrogates priming of CD8+ T cells by exogenous cell-associated antigens. Immunity **17:**211–220.
23. **Kaech, S. M., and R. Ahmed.** 2001. Memory CD8+ T cell differentiation: initial antigen encounter triggers a developmental program in naive cells. Nat. Immunol. **2:**415–422.
24. **Kerksiek, K. M., D. H. Busch, I. M. Pilip, S. E. Allen, and E. G. Pamer.** 1999. H2-M3-restricted T cells in bacterial infection: rapid primary but diminished memory responses. J. Exp. Med. **190:**195–204.
25. **Kocks, C., E. Gouin, M. Tabouret, P. Berche, H. Ohayon, and P. Cossart.** 1992. *L. monocytogenes*-induced actin assembly requires the *actA* gene product, a surface protein. Cell **68:**521–531.
26. **Lanzavecchia, A., and F. Sallusto.** 2001. Regulation of T cell immunity by dendritic cells. Cell **106:**263–266.
27. **Lauvau, G., S. Vijh, P. Kong, T. Horng, K. Kerksiek, N. Serbina, R. A. Tuma, and E. G. Pamer.** 2001. Priming of memory but not effector CD8 T cells by a killed bacterial vaccine. Science **294:**1735–1739.
28. **Lyons, A. B., and C. R. Parish.** 1994. Determination of lymphocyte division by flow cytometry. J. Immunol. Methods **171:**131–137.
29. **Mackaness, G. B.** 1962. Cellular resistance to infection. J. Exp. Med. **116:**381–406.
30. **Marrack, P., and J. Kappler.** 2004. Control of T cell viability. Annu. Rev. Immunol. **22:**765–787.
31. **Matloubian, M., M. Suresh, A. Glass, M. Galvan, K. Chow, J. K. Whitmire, C. M. Walsh, W. R. Clark, and R. Ahmed.** 1999. A role for perforin in downregulating T-cell responses during chronic viral infection. J. Virol. **73:**2527–2536.
32. **Mercado, R., S. Vijh, S. E. Allen, K. Kerksiek, I. M. Pilip, and E. G. Pamer.** 2000. Early programming of T cell populations responding to bacterial infection. J. Immunol. **165:**6833–6839.
33. **Murali-Krishna, K., J. D. Altman, M. Suresh, D. J. Sourdive, A. J. Zajac, J. D. Miller, J. Slansky, and R. Ahmed.** 1998. Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. Immunity **8:**177–187.
34. **North, R. J.** 1973. Cellular mediators of anti-*Listeria* immunity as an enlarged population of short-lived, replicating T cells: kinetics of their production. J. Exp. Med. **138:**342–355.
35. **Pamer, E. G.** 1994. Direct sequence identification and kinetic analysis of an MHC class I-restricted *Listeria monocytogenes* CTL epitope. J. Immunol. **152:**686–694.
36. **Pamer, E. G., J. T. Harty, and M. J. Bevan.** 1991. Precise prediction of a dominant class I MHC-restricted epitope of *Listeria monocytogenes*. Nature **353:**852–855.
37. **Pope, C., S. K. Kim, A. Marzo, D. Masopust, K. Williams, J. Jiang, H. Shen, and L. Lefrancois.** 2001. Organ-specific regulation of the CD8 T cell response to *Listeria monocytogenes* infection. J. Immunol. **166:**3402–3409.
38. **Sano, G., J. C. Hafalla, A. Morrot, R. Abe, J. J. Lafaille, and F. Zavala.** 2001. Swift development of protective effector functions in naive CD8+ T cells against malaria liver stages. J. Exp. Med. **194:**173–180.
39. **Stock, A. T., S. N. Mueller, A. L. van Lint, W. R. Heath, and F. R. Carbone.** 2004. Cutting edge: prolonged antigen presentation after herpes simplex virus-1 skin infection. J. Immunol. **173:**2241–2244.
40. **Tvinnereim, A. R., S. E. Hamilton, and J. T. Harty.** 2002. CD8+-T-cell response to secreted and nonsecreted antigens delivered by recombinant *Listeria monocytogenes* during secondary infection. Infect. Immun. **70:**153–162.
41. **Tvinnereim, A. R., and J. T. Harty.** 2000. CD8+ T-cell priming against a nonsecreted *Listeria monocytogenes* antigen is independent of the antimicrobial activities of gamma interferon. Infect. Immun. **68:**2196–2204.
42. **van der Most, R. G., A. Sette, C. Oseroff, J. Alexander, K. Murali-Krishna, L. L. Lau, S. Southwood, J. Sidney, R. W. Chesnut, M. Matloubian, and R. Ahmed.** 1996. Analysis of cytotoxic T cell responses to dominant and sub-

dominant epitopes during acute and chronic lymphocytic choriomeningitis virus infection. J. Immunol. **157:**5543–5554.
43. **van Stipdonk, M. J., E. E. Lemmens, and S. P. Schoenberger.** 2001. Naive CTLs require a single brief period of antigenic stimulation for clonal expansion and differentiation. Nat. Immunol. **2:**423–429.
44. **Vazquez-Boland, J. A., C. Kocks, S. Dramsi, H. Ohayon, C. Geoffroy, J. Mengaud, and P. Cossart.** 1992. Nucleotide sequence of the lecithinase operon of *Listeria monocytogenes* and possible role of lecithinase in cell-to-cell spread. Infect. Immun. **60:**219–230.
45. **Weston, S. A., and C. R. Parish.** 1990. New fluorescent dyes for lymphocyte migration studies: analysis by flow cytometry and fluorescence microscopy. J. Immunol. Methods **133:**87–97.
46. **Wherry, E. J., and R. Ahmed.** 2004. Memory CD8 T-cell differentiation during viral infection. J. Virol. **78:**5535–5545.
47. **Wherry, E. J., J. N. Blattman, K. Murali-Krishna, R. van der Most, and R. Ahmed.** 2003. Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment. J. Virol. **77:**4911–4927.
48. **Wherry, E. J., M. J. McElhaugh, and L. C. Eisenlohr.** 2002. Generation of $CD8^+$ T cell memory in response to low, high, and excessive levels of epitope. J. Immunol. **168:**4455–4461.
49. **Wherry, E. J., K. A. Puorro, A. Porgador, and L. C. Eisenlohr.** 1999. The induction of virus-specific CTL as a function of increasing epitope expression: responses rise steadily until excessively high levels of epitope are attained. J. Immunol. **163:**3735–3745.
50. **Williams, M. A., and M. J. Bevan.** 2004. Shortening the infectious period does not alter expansion of CD8 T cells but diminishes their capacity to differentiate into memory cells. J. Immunol. **173:**6694–6702.
51. **Wong, P., and E. G. Pamer.** 2001. Cutting edge: antigen-independent CD8 T cell proliferation. J. Immunol. **166:**5864–5868.
52. **Wong, P., and E. G. Pamer.** 2003. Feedback regulation of pathogen-specific T cell priming. Immunity **18:**499–511.
53. **Yewdell, J. W., and J. R. Bennink.** 1999. Immunodominance in major histocompatibility complex class I-restricted T lymphocyte responses. Annu. Rev. Immunol. **17:**51–88.

*Editor:* J. L. Flynn